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T-Cell Receptor Jβ1/Jβ2 Locus Rearrangements in an HTLV-1-Positive T-Cell Lymphoma With Complex Chromosomal Aberrations

M.J. Macera, P. Hyde, N. Peddanna, P. Szabo, S.K. Gogineni, and R.S. Verma

Division of Genetics (M.J.M., S.K.G., R.S.V.) and Department of Medicine (P.H., N.P.), The Long Island College Hospital-SUNY Health Science Center at Brooklyn, New York, and Division of Geriatrics, The New York Hospital-Cornell Medical Center, New York, New York (P.S.)

We report a case of human T-cell lymphotropic virus type 1 (HTLV-1)-infected adult T-cell lymphoma that has multiple chromosomal abnormalities, including the presence of an additional 7q22-36, which contains the locus of the T-cell receptor (TCR) beta chain gene. Specific TCR Jβ1/Jβ2 gene rearrangements were detected in both marrow and peripheral blood DNA, with evidence of further evolution of the transformed clonal population within the peripheral lymphocytes. To our knowledge, this is the first case in which gene rearrangements have been associated with additional TCR loci. Consequently, it is advised that every effort should be made to correlate chromosomal abnormalities with gene rearrangement by molecular methods. © 1996 Wiley-Liss, Inc.

Key words: chromosome 7, HTLV-1, T-cell lymphoma, FISH technique

INTRODUCTION

Human T-cell lymphotropic virus type 1 (HTLV-1) has been proposed to be a causative virus of adult T-cell leukemia/lymphoma (ATL) [1,2]. ATL is an aggressive malignancy of mature helper T lymphocytes [3]. One characteristic of this disease is the presence of numerous, often complex chromosomal abnormalities that have been difficult to identify by routine banding techniques [4–8]. A consistent chromosomal abnormality has not been reported. Shimoyama et al. [9] suggested that the prognosis of ATL does not depend on the type of chromosomal aberration. Nevertheless, those chromosomal regions known to contain genes participating in the T-cell response are frequently involved in the observed rearrangements [10–12].

Rearrangements within the T-cell receptor (TCR) genes in HTLV-1-associated ATL have been shown in the beta chain Cβ1, Cβ2, Vβ, Jβ1, and Jβ2 loci and the delta J chain loci [8,13–15] and alpha and gamma loci [16,17]. In one case [8], an expansion of T cells with a particular Jβ1/Jβ2 rearrangement was associated with the occurrence of unusual chromosomal abnormalities, although none of the aberrations involved 7q34-35, where the beta chain gene is located [18]. We report a case of HTLV-1, ATL with gene rearrangements within the T-cell Jβ1/Jβ2 loci and the presence of additional 7q34-35 material involved in an unusual translocation to chromosome 2q.

MATERIALS AND METHODS

Case Report

A-32-year-old Haitian male was referred for abnormal leukocytosis. On physical examination the patient was found to be febrile and anicteric with generalized lymphadenopathy and hepatosplenomegaly. Laboratory findings were: white blood cells 99×10^3 with differential of polymorphs 10%, lymphocytes 77%, monocytes 1% with atypical lymphocytes 12%. Hypercalcemia was noted. Lymphocyte immunophenotying of peripheral blood revealed 98% T cells with 96% CD4 cells. Bone marrow was moderately cellular and heterogenous in morphology, with 40–50% of the cellular elements lymphoid in nature and somewhat variable in size with slight nuclear irregularity. The lymphoid infiltration was throughout the mar-

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Address reprint requests to Dr. Ram S. Verma, Division of Genetics, The Long Island College Hospital, Hicks St. at Atlantic Ave., Brooklyn, NY 11201.

row specimen. The patient was confirmed HTLV-1 positive by enzyme-linked immunosorbent assay testing, and a diagnosis of adult T-cell lymphoma was made. He was also diagnosed to have pulmonary tuberculosis and was treated with a four-drug regimen. The patient deteriorated rapidly and expired on day 16 of hospitalization.

Cytogenetic Analysis

Bone marrow and unstimulated peripheral lymphocytes were cultured as previously described [19]. Initial karyotypic analysis was performed using QFQ- and GTG-banding techniques per the standard protocol [20].

FISH Hybridization

Slides were air dried 2 days prior to fluorescence in situ hybridization (FISH). The procedure used for each probe was as specified by the manufacturer. Briefly, for whole chromosome 1-, 2-, and 5-specific painting probes (WCP) (GIBCO-BRL, Grand Island, NY), chromosome 18-specific centromere probe (CEP) (Vysis, Downers Grove, IL), and WCP 7- and 18-specific probes (Oncor, Gaithersburg, MD), metaphases were denatured at 70°C in 70% formamide/2 × SSC (pH 7.0). Following dehydration in ethanol, the slides were hybridized overnight at 37°C in a humid chamber. The final stringencies of the post-hybridization washes were: for WCPs 1, 2, and 5 and CEP 18, 50% formamide/2 × SSC (pH 7.0), 2 × SSC/0.1% NP-40 at 45°C and for chromosome 7 and 18 probes, 1 X SSPE at 72°C. Chromosome 1-, 2-, and 5- and CEP 18-specific probes were spectrum orange fluorophore-labeled. The hybridization included unlabeled competitor human C_0t-1 DNA; DAPI/phenylenediamine dihydrochloride was used as a counterstain. Whole chromosome 7- and 18-specific probes were biotin labeled and detected with fluorescein-labeled avidin and propidium iodide counterstain.

Preparation of Cellular DNA

Total genomic DNA was isolated both from ammonium chloride (1.5 mM) treated lymphocytes prepared from fresh peripheral blood and from bone marrow. The cells were treated overnight at 37°C with proteinase K (200 μg) in 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1% sodium dodecyl sulfate (SDS), followed by several phenol/chloroform extractions, chloroform extraction, and ethanol precipitation.

Southern Blotting

Total genomic DNA was digested with the restriction endonuclease *Eco*RI using conditions recommended by the supplier. The resulting fragments were separated on 0.8% agarose gel and blotted onto a nylon filter using standard procedures [21]. ^{32}P-labeled plasmid probe Jβ1/Jβ2 (Oncor) in 50% formamide, 5 × Denhardt's solution, 5 × SSPE, 0.1% SDS, and 200 μg/ml single-strand salmon sperm DNA was hybridized to the filter for 48 hours at 42°C, followed by three washes in 2 × SSC, 0.1% SDS at 65°C and in 0.1 × SSC, 0.1% SDS at 60°C using standard procedures [22] and exposed to X-ray film for 3 days.

RESULTS

Cytogenetic analysis by GTG- and QFQ-banding revealed an abnormal 49,XY,+del(1)(p21),der(2)t(2;?7)(q37;?q22), +5, +mar karyotype in all of the dividing cells from the patient's unstimulated peripheral lymphocytes (50 cells) and 90% of the bone marrow cells (50 cells) (Fig. 1). FISH analysis with whole chromosome painting probes specific for chromosomes 1,2,5,7, and 18 confirmed the presence of an additional 1q, translocation of an additional 7q22-36 to chromosome 2q with loss of the terminal portion of chromosome 2, presence of a complete additional chromosome 5, and identification of a small marker chromosome as additional 5 and additional 18 material including the centromere, which was further clarified as 5p and 18q by GTG-banding (Fig. 2). The karyotype was modified to 49,XY,+del(1)(p21),der(2)t(2;7)(q37;q22),+5,+der(18)t(5;18)(p14;p11.1)del(18)(q22).

Bone marrow cells (Fig. 3, lane B) showed the two expected germline Jβ1/Jβ2 fragments at 11 and 4.2 kb, respectively. In addition to those fragments, two additional bands were seen at 5.1 and 7.3 kb, presumably representing the rearranged TCR of the transformed cell. A peripheral blood sample from the patient (Fig. 3, lane C) showed the expected germline fragments at 11 and 4.2 kb, the rearranged fragments at 5.1 and 7.3 kb, and a novel fragment at 2.5 kb. The intensities of hybridization to the Jβ1/Jβ2 fragments differed in the bone marrow and peripheral blood samples.

DISCUSSION

In recent years, characterization of highly complex chromosomal abnormalities by FISH techniques has become a routine approach, especially for deciphering unusual anomalies. In the present case, identification of the marker chromosome as being derived from additional 5p and 18q segments as well as the additional 7q material could not have been done by conventional cytogenetic methods. The involvement of 7q material suggested that examining the structure of the TCR beta gene may provide some insight into the mechanisms leading to HTLV-1-induced chromosome aberrations. We previously documented the occurrence of unusual chromosomal abnormalities not involving 7q with the appearance of TCR clonal selection of Jβ1/Jβ2 gene rearrangements [8]. This is the first case in which additional TCR loci have been seen.

The most intriguing aspect of this case is that the DNA

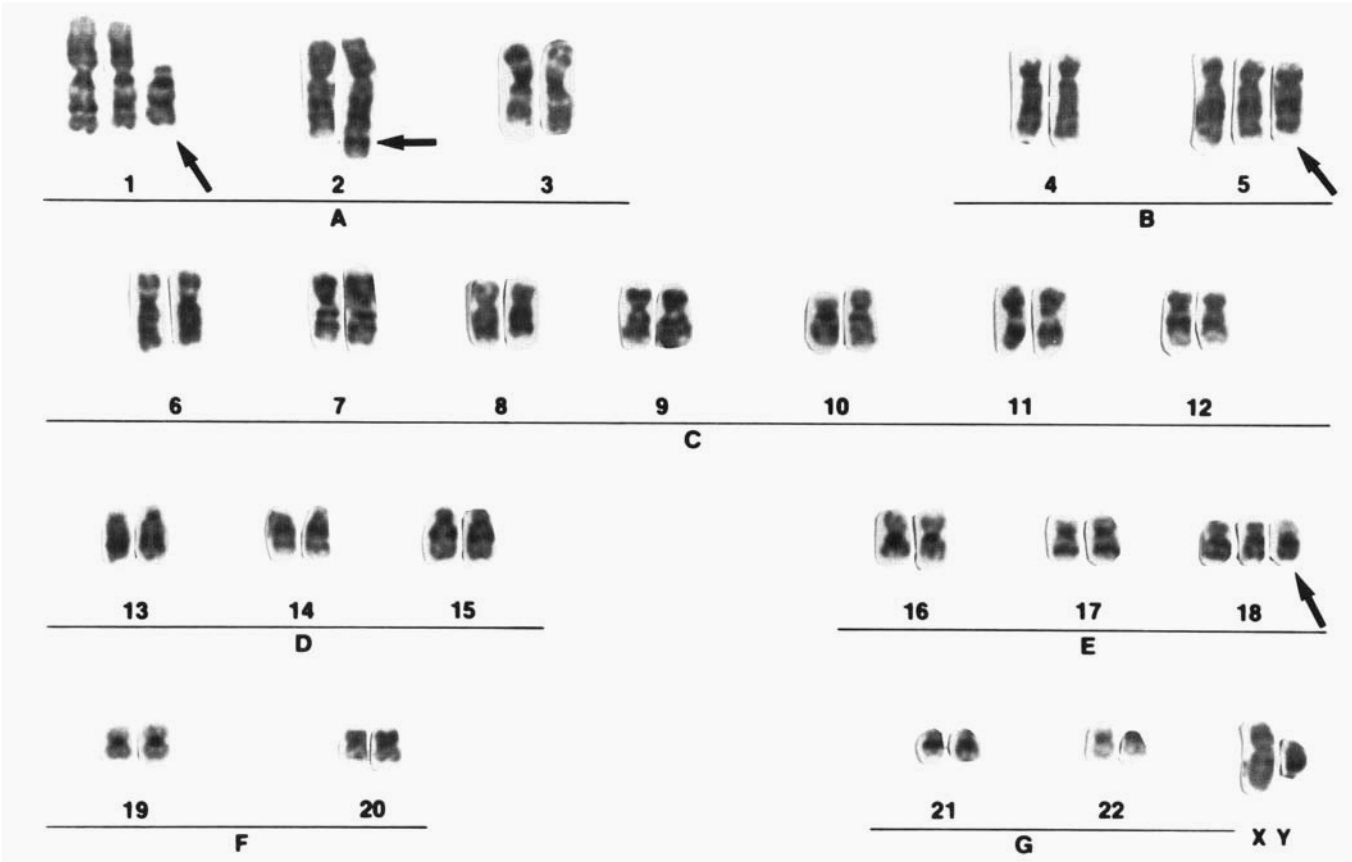

Fig. 1. GTG-banding pattern of chromosomes with abnormal 49,XY,+del(1)(p21), der(2)-t(2;7)(q37;q22),+5,+der(18)t(5;18) (p14;p11.1) del(18)(q22) (see arrows).

from peripheral blood (Fig. 3, lane C) shows not only the same Jβ1/Jβ2 gene rearrangement of the functional TCR beta chain gene as the bone marrow cells with bands at 7.3 and 5.1 kb (Fig. 3, lane B), but also an additional 2.5 kb fragment. HTLV-1 is known to infect mature T-helper cells; in this patient, the transformed T cell infiltrated the bone marrow and accounted for 40–50% of the cellular bone marrow element. HTLV-1 infiltration of bone marrow has been demonstrated [12]. The TCR Jβ1/Jβ2 gene fragments seen in bone marrow DNA reflects infiltration of the HTLV-1-infected T cells. Since normal noninfected cells would only yield the expected germline fragments and there is also a normal allele in the HTLV-1-infected clone, one would expect the majority of the Jβ1/Jβ2 DNA to show the germline pattern. In fact, densitometry analysis suggests that about 50% of the bone marrow cells are of transformed origin, in agreement with the immunophenotyping data.

Immunophenotyping analysis of the peripheral blood showed 98% of mononuclear cells were T cells, with 96% of the population CD4 helper cells. Thus, it would appear that the blood DNA (Fig. 3, lane C) primarily represents the HTLV-1-infected clonal cell population. The observed reduction in germline fragments compared with the bone marrow is to be expected. Both the infected marrow population and the peripheral blood population have an additional copy of the TCR beta locus translocated to chromosome 2. From densitometry readings, it appears that the 7.3 and 5.1 kb fragments are twice the intensity of the 4.2 kb germline fragment. This would suggest that the additional TCR locus translocated to chromosome 2 is a duplication of the functionally rearranged locus, not the normal germline allele. The additional 2.5 kb fragment seen in the peripheral blood, but not in the marrow, appears to have arisen from the 7.3 kb fragment of the rearranged gene, since densitometry readings suggest that the 7.3 kb fragments in the marrow is more intense than in the blood, relative to the 5.1 kb fragment. The 2.5 kb fragment probably arose from a break within the 7.3 kb fragment on one of the two rearranged loci of the mature infected clone. Alternatively, it is possible that the 2.5 kb fragment arose from one of the germline loci; the fragment intensity at 11.0 kb is also reduced in the blood, which may have occurred during an additional breakage in the translocated gene. No evidence of any new gene rearrangement was detectable cytogenetically.

In the unstimulated peripheral blood mitotic cells, only

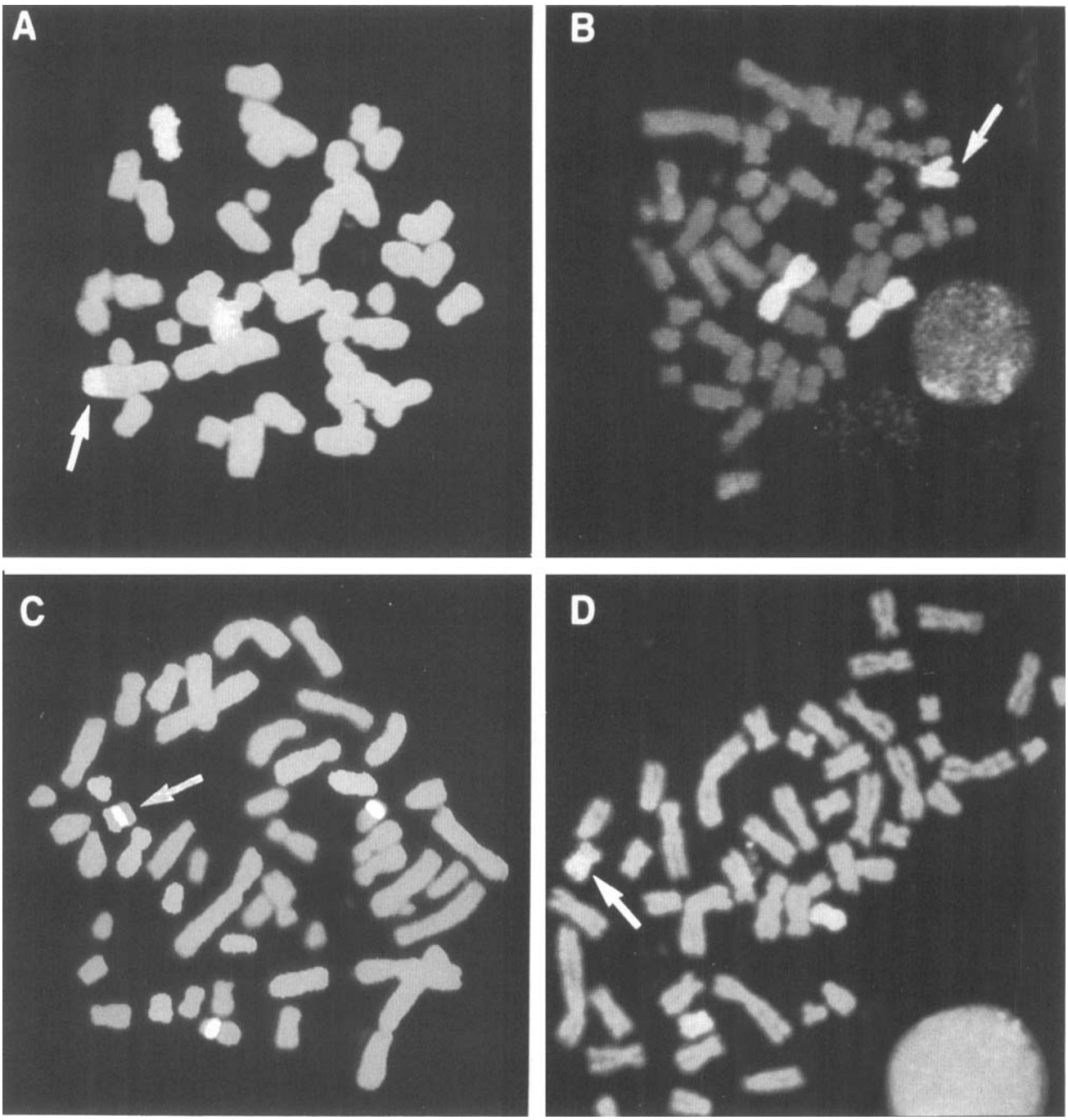

Fig. 2. FISH technique showing (A) whole chromosome 7-specific probe (WCP), (B) WCP 1, (C) chromosome 18 centromeric-specific probe, and (D) WCP 18 probe. The derivative chromosomes are marked by the arrows.

the abnormal clone was observed. Mitotic bone marrow cells showed approximately 10% normal 46,XY cells, with the remaining dividing cells having the same abnormal chromosome complement as the blood. The possibility that the 2.5 kb fragment is derived from a second HTLV-1-infected T cell is remote since the chances of a separate cell arising with similar TCR rearrangements are negligible, and no mitotic cells with other chromosome abnormalities were detected. The reduction in the intensity of the 7.3 and 11.0 kb fragments in concert with the appearance of the 2.5 kb fragment suggests that clonal expansion of a second aberration involving the TCR beta gene of the original infected population within the peripheral blood has occurred. In this case, the Southern blot data suggest that the original transformed T-cell population contained two identical rearranged loci and a single germ-

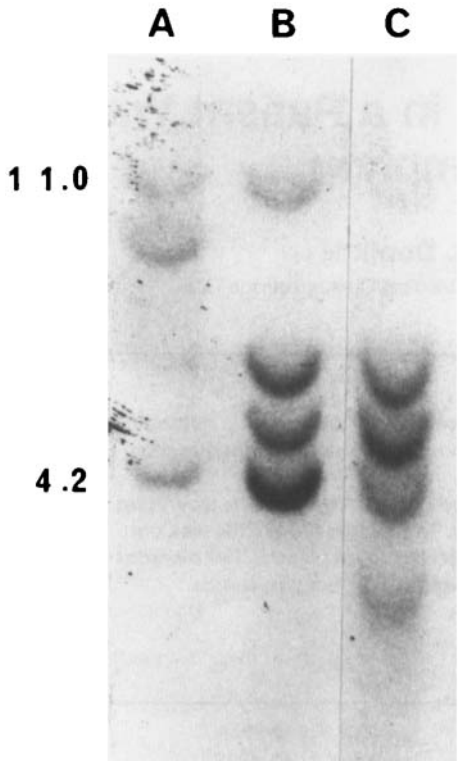

Fig. 3. *Eco*R1-digested DNA from (lane A) T-cell line, (lane B) bone marrow, and (lane C) peripheral blood from the patient. The germline Jβ1 and Jβ2 loci are lcoated at 11.0 and 4.2 kb, respectively (see text).

line gene. It appears likely that the additional TCR beta locus that has been translocated to chromosome 2 originated from the rearranged chromosome.

It is becoming apparent that the numerous chromosomal aberrations associated with HTLV-1-infected ATL are secondary events resulting from the disease process rather than an etiology. Furthermore, chromosome analysis, complemented with TCR loci-specific studies, may lead to a better understanding of the mechanism(s) causing HTLV-1-induced anomalies. This approach might further enhance our knowledge of the genetic basis of this virulent fatal disease.

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